

## CHAPTER 1

# Sampling theory and practice

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## Introduction

This chapter deals with the need to sample insects, the theory underlying sampling, the need to calibrate samples, and the design of sampling programs, and it evaluates the use of different sampling techniques.

## Why sample?

Sampling is a scientist's way of collecting information, and the majority of sampling is undertaken to answer specific questions. This was not always the case. Sampling as we know it was first done in a haphazard manner and bore little relation to what we would call sampling today. The first samples taken were basically a by-product of the desire of natural historians to collect information about the world around them.

## A brief history of information collection

The history of information collection can be classified into three main stages. There is a little overlap, but in the main we can recognize three separate phases.

### 1 The collectors

This can be classified as the pin, stuff, and draw era. As travel became relatively safer and people became more interested in what lay beyond their horizons there was a rapid expansion both in the number of naturalists traveling to other continents and in the number of people employed by naturalists to collect and return specimens to Europe. Drawing was also a popular activity and to a certain extent filled the niche now occupied by photography. Many ships' officers were accomplished amateur artists and many had an interest in the flora and fauna of the countries they visited. This phase resulted in the acquisition of many thousands of specimens of plants and animals, either stuffed, pickled,

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pressed, or pinned, accompanied by many sketches of the organisms in their native settings, although as the majority of the artists had no scientific training these drawings and paintings sometimes bear only a passing resemblance to reality.

Although this resulted in the garnering of many examples of plants and animals there was little knowledge of the biology or ecology of the organisms. This led to a great deal of confusion, particularly in the field of entomology where the sometimes complicated life cycles such as those occurring in dimorphic and polymorphic species such as aphids led to the misclassification of many species. For example, several aphid species were classified as being more than one species, depending on which host plant they were removed from or depending on which stage of the life cycle they were in at the time of their collection. Other similar mistakes occurred in the Lepidoptera where confusion over the identity of members of several mimic species lasted for some time until the larval stages were recognized. Ladybird beetles such as *Adalia bipunctata* and *Adalia decempunctata*, now well known as being extremely variable in their different color forms were also once misidentified as separate species until their life histories were fully elucidated.

### 2 The observers

There were of course some collectors who also collected observations. Many natural historians, as well as having a keen eye for the chase and for sketching, also felt the need to observe the behavior of the animals that they were collecting. These are exemplified by Darwin and Fabre who, as well as making detailed collections of specimens, also spent many hours observing and recording patterns of behavior. These observations provided plenty of information on the biology of the species, but as much of it was centered on individuals and their interactions with other individuals of the same species did not provide a great deal of information on their place in ecosystems, did not always provide accurate information about mortality factors and was confounded by a great deal of unrecognized environmental “noise.”

### 3 Experimental/controlled sampling

The next great step forward in the field of information collecting was the use of experimental studies in controlled conditions. For example, by studying the biology of an insect in the laboratory, it is possible to obtain detailed knowledge of life history parameters such as fecundity, longevity, etc., and it is also possible to assign specific values to mortality factors, albeit in a far from natural environment. The main drawback of this type of study is that environmental variability is lost and the natural impact of mortality and natality factors is compromised.

The best option is to combine laboratory methods and natural conditions, and

to do experimental and manipulative work in the field. The need to obtain accurate estimates of animal numbers in the field led to the development of the theory of sampling and, incidentally, to the use of statistics in the biological sciences.

### **Estimating abundance and predicting population dynamics**

The major use of sampling in entomology is to determine the number of insects in a given area or location, usually for pest control or conservation purposes. The other main reason for wanting information on insect numbers is to increase our understanding of the population dynamics of the insect(s) in question and to make predictions of their future abundance.

Before one can make a prediction, one needs to know how many insects there are in the first place. This is equally true, whether one is going to control a pest or to conserve an endangered species. It is not a sound practice (although some modelers do it) to conjure a number out of thin air. There is also a need to know what factors affect those numbers. There are basically six facts about the population of an insect that are required before sensible predictions of the population dynamics can be made:

- 1** density — an expression of the species' abundance in an area;
- 2** dispersion (distribution) — the spatial distribution of individuals of a species;
- 3** natality — birth rate;
- 4** mortality — death rate;
- 5** age structure — the relative proportions of individuals in different age classes;
- 6** population trend — the trend in the abundance of the study species.

It is only from this sort of information that one can start to make some sort of inferences about the population dynamics of the insect. The only reliable way to obtain this type of information is to sample.

### **Sampling methods**

To sample an insect requires both a sampling technique and a sampling program. These are different things, although it is noticeable that even in the scientific literature the two terms are quite often used interchangeably.

### **Sampling techniques**

A sampling technique is the method used to collect information from a single sampling unit. Therefore the focus of a sampling technique is on the equipment and/or the way the count is accomplished.

### Sampling programs

A sampling program, on the other hand, is the procedure for employing the sampling technique to obtain a sample and make an estimate. Sampling programs direct how a sample is to be taken, including sampling unit size, number of sample units, spatial pattern of obtaining sampling units, and timing of samples.

Before, however, one starts to think about either the sampling unit or the sampling program it is necessary to know something about the insect that is going to be sampled.

An important starting point is to find out about the life cycle and biology of the insect and especially about where it is likely to be found. There is no point in sampling terrestrial habitats for something that lives in water. Some insects have marked changes in distribution during the course of a year, so it is important that this is taken into account before any sampling program is undertaken. For example, the bird cherry aphid *Rhopalosiphum padi* lives on grasses in summer and on the bird cherry tree *Prunus padus* in autumn, winter, and spring (Dixon & Glen 1971). For those insects that show seasonal changes in habitat use, it is essential to know when the changeover from habitat to habitat takes place, at least approximately. Sampling will of course have to be conducted in both habitats for some period of time to pinpoint this changeover. Thus, a good knowledge of the biology and ecology of the insect is very important. Another important consideration is the likely cost of the sampling in terms of both time and money.

### Deciding on the approach

#### Sampling tools/techniques

There are a number of tools that can be used to sample insect populations. One can sample aerially, for example using suction traps. These are used throughout Britain by Rothamsted Insect Survey (Knight et al. 1992) and in many other parts of the world. They are primarily used to trap aphids, and sample at two standard heights, 1.2 m and 12.2 m. Sticky traps, either with or without attractants, can be used for almost anything that flies and is too weak to get off the sticky board. Light traps are also commonly used to sample aerial populations, although the insects mainly caught are night-flying Lepidoptera. There are various intercept traps that are used to catch beetles, flies, aphids, and other insects, such as yellow water traps, Malaise traps and window traps. These are discussed further in other chapters. It is useful to note that the range of technology is quite vast. A great deal of effort can go into the design and evaluation of traps, and this is often an essential part of the design of a sampling program. For example, some insects are more readily caught by certain types of trap (Heathcote 1957,

Niemelä et al. 1986). The behavior of the insect will largely determine the type of trap or sampling tool used (see later chapters).

### **Passive traps versus active traps**

Sampling and trapping techniques can broadly be classified into two types—passive or active.

A passive trap is one that should be neutral and depends entirely on chance. An active trap depends on the behavior of the insect but takes advantage of the behavior and attracts the insect to the trap by chemical lures, baits, or even color—all of which can be varied to give different trapping efficiencies and targets (Finch 1990).

### **What is the advantage of a passive trap over an active trap?**

Passive traps allow unbiased estimates of insect populations because the insects are neither attracted nor repelled by the traps. For example, although aerial suction traps are powered by a motor and draw air into the collecting tube they are essentially passive in action as they depend on the insect flying into the ambit of the trap and do not depend on it being attracted to the area. A big drawback to the use of passive traps is that they are not very useful at low densities. This is a particular problem when programs have been designed to monitor the abundance or occurrence of pests—for example insects on quarantine lists. In those cases an attractant trap is a much better alternative as they are better detection tools. They do, however, give a biased estimate of the density per unit area and conversion factors then have to be applied. Thus, when using attractant traps, particularly if they are being used to obtain population estimates, it is vitally important to know over what range the trap is effective and whether there are directional as well as distance effects.

### **Direct habitat sampling**

Sometimes, particularly if one is working with a pest species, the most useful method of sampling is one that estimates the population size in the habitat—e.g. a crop or nature reserve. Indirect methods of sampling—e.g. aerial sampling with a suction trap or pan trapping in a field—only indicate what is present in the area, and do not tell you what is actually on the plant or in the soil. It will tell you what is there and gives some idea of whether there are many or few, but unless it has been backed up by calibration studies it does not tell you how many insects there are per plant or per unit area of habitat, or whether they are actually present on the area that you are concerned with; they may just have been en route somewhere when they were caught. This is particularly true of migratory insects.

## What methods are available and what determines their use?

Particular methods are dealt with in the following chapters. Here, we consider the rationale behind the selection of available techniques. When sampling on the ground there are a number of methods available. Quadrats may be used for some insects—e.g. predatory surface-active beetles, aphids on plants, etc. However, searching a surface quadrat is no good for cryptic, soil-dwelling nocturnal insects such as the large pine weevil *Hylobius abietis*. Whole-plant searches or part-plant searches are also useful, and if the insects are relatively sedentary can give good population estimates. Pitfall trapping is useful for surface-active insects, particularly those active during the night, (see Chapter 3). Soil extraction methods can be used for soil or root-dwelling species (see Chapter 2).

## Destructive versus non-destructive sampling

If whole plants or small areas of habitat are to be sampled, two approaches can be used—destructive and non-destructive. Destructive sampling involves the removal of the sample unit for later assessment (see below), whereas with non-destructive sampling the sample unit is searched or sampled *in situ*.

Both these approaches have their merits and disadvantages. For example, suppose you are counting aphids on a plant. You could sample destructively—i.e. remove the plant or part of the plant from the ground or from the main stem, and bring it back into the laboratory. Alternatively, you could sample non-destructively—for example, examine 100 leaves and record what is found.

Destructive sampling is more accurate as the insects are less likely to escape during the counting process. One cannot, however, go back and sample the same plant or area again. This is a particular problem if there are only a limited number of plants to begin with, or if the habitat type is rare and easily disturbed. If one is sampling from a large number of uniform plants such as a field of leeks or a forest plantation, destructive sampling may be a useful technique. A disadvantage of destructive sampling is that it is more time consuming, and is thus not useful in situations where a quick estimate of insect numbers is required, say for a control operation. It is possible with destructive sampling, however, to postpone sampling by storage, be it in the freezer or in some sort of preservative. This is particularly useful in those situations where a large number of samples have to be taken in a limited time period and where there is no need for a swift result. It means that the actual counting of the insects can be saved for a less busy time of year—e.g. the winter.

Non-destructive sampling, on the other hand, does allow re-sampling of the plants and habitats on a frequent or regular basis. This is very useful in sensitive areas and when local population dynamics are being studied. Non-destructive sampling tends to be quicker than destructive sampling and causes less disturbance to the habitat. It does however depend on the insects being relatively sedentary or slow to respond to disturbance. Thus the counts will tend to be

underestimates. To counter this, as non-destructive sampling is fairly quick, more samples can be taken, although this does not entirely solve the problems of underestimation.

### How many samples?

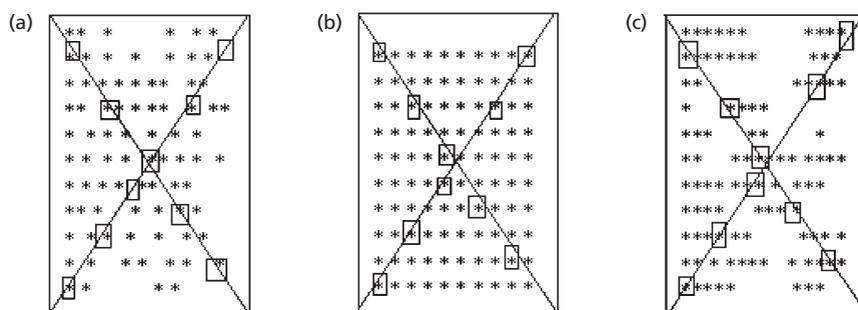
There are a number of factors that determine the number of samples that are taken. The first requirement is to be sure that the sample taken is representative of the population that is being sampled. To ascertain this it may be necessary to perform stratified sampling. It is not always safe to assume that insects are systematically distributed. A number of different distributions are possible. The population could be randomly distributed, uniformly distributed, or even in an aggregated (clumped or contagious) distribution (Fig. 1.1). These factors all need considering. It is possible to determine what distribution the population has by using the following approach.

### Variance—mean ratios

The dispersion of a population determines the relationships between the variance  $s^2$  and the arithmetic mean  $\mu$  thus:

- 1 random distribution — the variance is equal to the mean —  $s^2 = \mu$ ;
- 2 regular (uniform) distribution — the variance is less than the mean —  $s^2 < \mu$ ;
- 3 aggregated (clumped or contagious) distribution — the variance is greater than the mean —  $s^2 > \mu$ .

The distribution of the organism can have a marked influence on the way in which you might sample. Take, for example, a site in which the organism you are going to sample has a soil-dwelling pupae. The easiest approach is to do a simple line transect from one corner of the field to another, or if you are



**Fig. 1.1** Sampling different distributions with a common sample plan: (a) random, (b) uniform or regular, and (c) aggregated distribution. Note that the values returned, even in this simple example, are very different for the aggregated distribution.

concerned about the slope or topography you might do another transect across from the other two corners in the form of an X. Depending on the distribution of the organism you may get totally different answers (Fig. 1.1).

### Stratified sampling

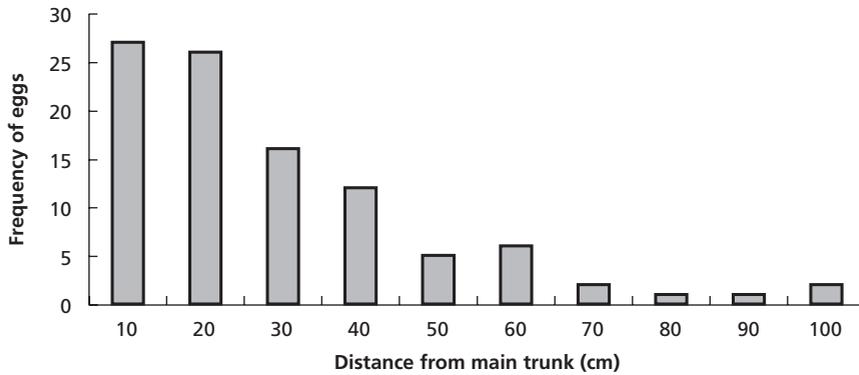
Suppose we know that the population we are going to sample varies systematically across the area we are going to sample.

We may know, for example, that the insect does not occur in high densities in particular areas—e.g. where there are lots of stones—but does occur in high numbers in areas where there is a lot of sand. It may even be something more specific: for example, if we were sampling trees for insects, we might know that the distribution of the insect within the crown of the tree is not uniform. The pine beauty moth, for example, lays most of its eggs in the upper third of the tree, so one can get a good estimate of the population by just counting eggs found on the first five whorls and then either multiplying up or just taking the figure obtained to be representative of the population (Watt & Leather 1988a). It really depends on what one is sampling for. If one is sampling for predictive purposes than the first five whorls is good enough; on the other hand, if the sampling is part of a detailed population study, then the sampling needs to be more thorough and to take more account of the distribution of the organism. Thus, for the pine beauty moth, a branch is taken from every other whorl, the number of branches per whorl counted, and the counts are then multiplied up accordingly. If one had a very large scale study, one might just take a third-whorl branch at random and multiply up from there (Leather 1993). Of course one would have to have done some whole-tree sampling first to determine what all the various multiplication factors were going to be. For example, with winter moth eggs on Sitka spruce there is a marked difference in egg distribution, not just in relation to tree height, but also within the branches (Watt et al. 1992) (Fig. 1.2). One could therefore work out various sampling schemes to use.

In essence, though, before a sampling scheme can be devised, one needs to do some preliminary sampling to get a feel for what number or size of sample one will require.

In general, the more samples that are taken the more precise the population estimates will be. However, time and expense are always constraining factors. Thus the usual approach is to decide on the lowest number of samples that can be taken to achieve a reasonable population estimate within the error limits set (Box 1.1).

One should make such calculations throughout the season. So for example if you are sampling cereal aphids at the beginning of a season when numbers are low you would start with a thousand tillers per field, and make adjustments as the population rises—but never below 100 tillers per field. There is usually a minimum value that the sampler never falls below and a maximum that



**Fig. 1.2** The distribution of winter moth eggs along Sitka spruce branches. Data from Watt et al. (1992).

**Box 1.1 To calculate the number of samples required**

$$n = (\sigma/\mu) \times cv)^2$$

where  $n$  is the number of samples required,  $\sigma$  (sigma) is the variance,  $\mu$  (mu) is the unknown mean of the population, and  $cv$  is the coefficient of variation of the mean which in turn is defined as

$$cv(X) = \sigma/\mu$$

For practical purposes one needs to collect a series of samples and make preliminary estimates of the mean ( $X_e$ ) and the variance ( $Se$ ) and then use this formula

$$n = (Se/X_e \times cv)^2$$

is never exceeded: these are determined by time and the requirement for accuracy. (For a number of case studies see Chapter 9.)

## Sampling concepts

### Choosing a sample unit

*What is a sampling unit?*

A sampling unit is a proportion of the habitable space from which insect counts are taken. The units must be distinct and not overlap. A sampling unit can be very variable in form. For example, it could be direct counts of all the caterpillars

**Box 1.2 Possible types of information required from a sampling scheme**

- 1 Estimates of population density per unit area
- 2 Assessments of percentage infestation or parasitism
- 3 Estimation of damage per unit area
- 4 Absolute population counts

in 1 m<sup>2</sup> of cereal field, or it could be 20 sweeps of a sweep net down a row. More usually, a sample unit is more easily measurable, e.g. a quadrat or template of a known area.

*What determines the choice of a sampling unit?*

The choice of sampling unit is dependent on what information is required. In an agricultural system the grower most frequently wants to know whether a particular insect pest has reached a threshold level which requires action (e.g. spraying), or what proportion of the crop is infested. In other circumstances (e.g. a population study) the observer may be more interested in the number of insects per given unit area (Box 1.2).

**Criteria for sampling units**

Sample units must meet a number of criteria if they are to be useful.

*1 Each sample unit should have an equal chance of selection*

This is where it is important to know what type of distribution individuals within the population display. Unfortunately using a totally random sampling scheme in some situations, even agricultural, can be too expensive in terms of time. Certainly in some situations—e.g. in a dense forest—it may not be logistically possible to apply a totally random sampling pattern. Therefore most fields and plots are sampled on a prearranged pattern—e.g. two X's, a V, a W, or whatever, with the samples collected along the transects. A degree of randomization can then be introduced, for example by varying the distance between sampling stations or by taking samples from either side of the transect on a random basis. It is important to avoid bias when sampling. This is particularly easy to introduce when sampling in crops. It is difficult to avoid selecting the leaves that look infested, e.g. discolored or curling. In cases like that, an element of chance should be built into the process when arriving at the sample station—e.g. take the first plant on the left, or throw a quadrat to standardize the sampling unit.

*2 The proportion of an insect population using the sample unit as habitat should remain constant throughout the sampling event*

If for example the insect moves around depending on time of day, then your population estimates will vary accordingly — e.g. the beet armyworm *Spodoptera exigua* is phototactic so sampling at midday will produce lower counts than during twilight or dawn as the larvae move into the ground or center of plants at high light intensities. The large pine weevil *Hyllobius abietis* is another example — it is night active so sampling should be done at the same time each day to keep things constant. Sampling should therefore be planned to take these factors into account.

*3 There should be a reasonable balance between the variance produced when data are collected from a given sample unit and the cost (time, labor, or equipment) in assessing that unit*

Generally a preferred sample unit would be the minimum size which would allow an adequate number of replications on a given date to produce averages with meaningful variance. Sampling all the leaves on a plant would provide very accurate information on that plant but as one would only be able to sample a few plants then the population estimate for the site would be extremely poor. Incidence counts are also useful (Ward et al. 1985). These rely on intensive sampling over a number of seasons so that one has a robust relationship between the numbers of insects present and the infestation rate of those plants. This is a very useful technique for non-experts such as farmers. It is however, not a feasible option unless the preliminary studies have been completed. Caution should also be exercised with this method as the relationship between incidence and population can change.

*4 Whenever possible or practical the sample unit should be as near as possible to the natural habitat unit*

In other words the area within which the insect is likely to spend most of its time in a given developmental stage — e.g. a cereal plant for an aphid, a leaf on a tree for an aphid or leaf miner, a branch for a defoliating caterpillar, and so on. Insects without discrete habitats — e.g. soil dwellers, predatory beetles, etc. — are somewhat more problematic and in such cases it is probably wise to rely on random quadrats etc.

*5 A sample unit should have stability*

Or, if not, then its changes should be easily and continuously measured — e.g. the number of shoots in a cereal crop.

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6 *The sampling unit must be easily delineated or described*

For example, buds on a branch, leaves, or plants, or quadrats of standard size.

7 *Ideally a sample unit should be able to be converted to some measure of unit area*

Thus it is important to count the number of trees in a compartment or plants in a field, etc., and then to be able to convert the counts obtained to numbers per  $m^2$ , for example (Box 1.3). What conversion is used, however, is less important than the fact that a conversion of some type is required in order to compare the density of different stages of the same insect species. This is essential if the mortality occurring between different stages is to be estimated.

8 *The number and location of sample units should be selected according to the purpose of the sampling*

Thus one could just sample the ears of cereal plants if one was interested in *Sitobion avenae* for prediction purposes (George & Gair 1979), whereas whole-plant counts would be needed for population estimates (Leather et al. 1984).

**Box 1.3 Sampling the pine beauty moth**

The pine beauty moth *Panolis flammea* has a typical univoltine lifecycle. The adult lays eggs on pine needles which hatch into larvae that pass through five instars whilst feeding in the canopy. The fifth-instar larva stops feeding and passes into a pre-pupal stage that spins to the ground, burrows into the litter layer, and pupates (Watt & Leather 1988b).

Sampling is carried out at all stages of the life cycle. Although each sampling technique gives a different output, they are all easily converted to a common measure, in this case individuals per square meter.

Stage	Method	Output	Conversion
Adult	Pheromone trap	Males per trap	Calibrated to area covered by trap
Eggs	Needle counts	Eggs per whorl	Converted to projected area covered by tree
Larvae	Funnel traps	Head capsules per funnel	Collecting area of funnel known
Pre-pupae	Basin traps	Pre-pupae per basin	Collecting area of basin known
Pupae	Soil sample	Pupae per 15 $cm^2$	Converted to per $m^2$ measure

### Informed sampling and collecting

As one works more and more with insects, one gains a knowledge or feeling of where to find particular groups or species. Although this is not strictly sampling, it does help inform the sampling process, and when one requires insects to start cultures or laboratory and field experiments it is certainly useful to be able to locate relatively large numbers of specimens quickly and easily.

In general, insects are small and relatively fragile, their reproductive and development rates are highly influenced by environmental factors, in particular temperature, and many of them, especially in their larval stages, are likely to feature in the diets of birds and other vertebrates as well as arthropod predators. This tends to mean that insects, except for the brightly colored highly mobile species such as butterflies, are more likely to spend most of their day in sheltered or concealed habitats, and in fact many insect species have taken this to the extreme and spend much of their life cycle living and feeding within plant parts — e.g. gall insects, leaf miners, bark beetles. Therefore, if looking for a ready supply of various insect species, dense clumps of grass, piles of leaves, under rocks and stones, in tree hollows and crevices, under loose bark, under logs, or even in fungi, will prove rewarding sites to search. Very dry habitats are unlikely to yield large numbers of individuals or species, but a moist, sheltered hollow under a broad-leaved tree is a sure source of a myriad of different species, albeit not all insects.

Insects, particularly herbivorous ones, are of course closely associated with their host plants, and certain times of year and sites on the plant are more likely to yield results than others. Certain plant species naturally potentially harbor more insect species than others. Oaks, willows, and birches are natural hot spots for insects of all descriptions from bark-dwelling Psocoptera to gallers, miners, general defoliators, and sap suckers. Many herbivorous insects depend on a ready supply of nitrogen to enable them to develop quickly at the beginning of the year. Check meristems, developing buds, young shoots, and flower buds for caterpillars and sap suckers. Birch aphids *Euceraphis punctipennis* closely follow growing shoots. Curled or distorted leaves are often signs that sap suckers or leaf tiers are in the vicinity, although be warned that these deformations will persist long after the insect has completed its life cycle and departed. Similarly, sooty mould, sticky leaves, and silken threads are often signs that aphids, other sap suckers, and web-spinning Lepidoptera are or have been present. Swellings on stems and sap and resin flows may also indicate the presence of stem borers, gallers, and bark beetles.

In temperate parts of the world insects spend a large proportion of their life cycle overwintering (Leather et al. 1993). Many have behavioral adaptations that cause them to seek out specific overwintering sites — e.g. negative phototaxis that causes them to search for dark crevices or thigmotactic responses that make them aggregate. If looking for ladybirds during the winter, it is often useful to look under loose bark, under window sills, or even on fence posts. Aggre-

gations often form in such situations. If your insect overwinters in the soil, avoid wet places and look for well-drained sites, preferably under trees rather than in the open. Overwintering is a costly business and insects attempt to minimize costs by overwintering in sites where the soil is unlikely to freeze, below about 10 cm depth. During winter, searching under hedges, in the middle of rotting logs, and in dense clumps of grass is also likely to repay one's efforts.

In general, think shelter, food, and protection and you are likely to find some insects in a relatively short space of time.

## Conclusions

In this chapter we have tried to give an overview of the philosophy of sampling, the rationale behind the choice of sample unit and technique, and some pointers towards what is the best approach to use in particular situations. We have not provided detailed mathematical and statistical formulae or numerous worked examples. Those wishing to acquire more of the mathematical background should consult two excellent textbooks that provide a wealth of such information, Southwood and Henderson (2000) and Sutherland (1996). Chapters within this book provide more specific mathematical and theoretical approaches for specific cases, but in the main deal with the practicalities of sampling either in specific habitats or with problematic guilds or groups.

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